

A theoretical study of the conformational behavior of analogues of α -L-rhamnose-1-phosphate[☆]

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Abstract—The conformational behavior of methyl(2-*O*-methyl- α -L-rhamnopyranosyl)phosphate, together with a group of potentially more stable analogues, was investigated through a DFT approach at the B3LYP/6-31G(d) level; the energy of all the optimized structures was recalculated using a continuum solvent model, C-PCM, choosing water as the solvent. The compounds exhibited several, sometimes tenths of populated conformations so that the overall properties of flexibility and mobility were evaluated. The analogue in which the pyranose oxygen atom is replaced by a methylene group emerges as the best candidate as a mimic of the reference 1-phosphate, in spite of the fact that it lacks the anomeric and *exo*-anomeric effects. The other analogues result poorer mimics because of a conformational equilibrium at the pyranose ring or of an excessive rigidity of the aglycone moiety.

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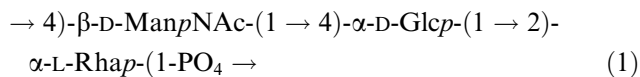
1. Introduction

Capsular polysaccharides of pathogenic bacteria are responsible of the immunogenic activity and, as a consequence, there is interest for their use as vaccines. These polysaccharides consist of mono-, di-, or trisaccharidic repeating units linked through a phosphate bridge that connects the reducing end of one unit to the following unit. Oligosaccharides constituted by a small number of repeating units, and even a single phosphorylated repeating unit, could be still provided with immunogenic activity and this fact prompted several research groups to perform the synthesis of repeating units of capsular polysaccharides to evaluate the role played by different structural factors on the activity.

However, these syntheses are often rather complex so that it is advisable either the realization of simpler

synthetic paths or the design and synthesis of simplified analogues still provided with immunogenic activity. When the synthetic targets include the phosphate group some more drawbacks derive from its presence because of the relatively low stability of glycosyl phosphates as well as the difficulties connected with their synthesis.

In the last years, the trisaccharide corresponding to the repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* type 19F has been the goal of synthetic efforts and some schemes for its preparation have been realized and published in literature.^{1–7} The structure of this capsular polysaccharide is represented by formula 1.



The phosphate group is α -linked to the anomeric position of L-rhamnose and this arrangement is a potential reason of instability during the synthesis of the repeating unit. In a synthetic plan, more stable compounds could derive from the replacement of one of the oxygen atoms

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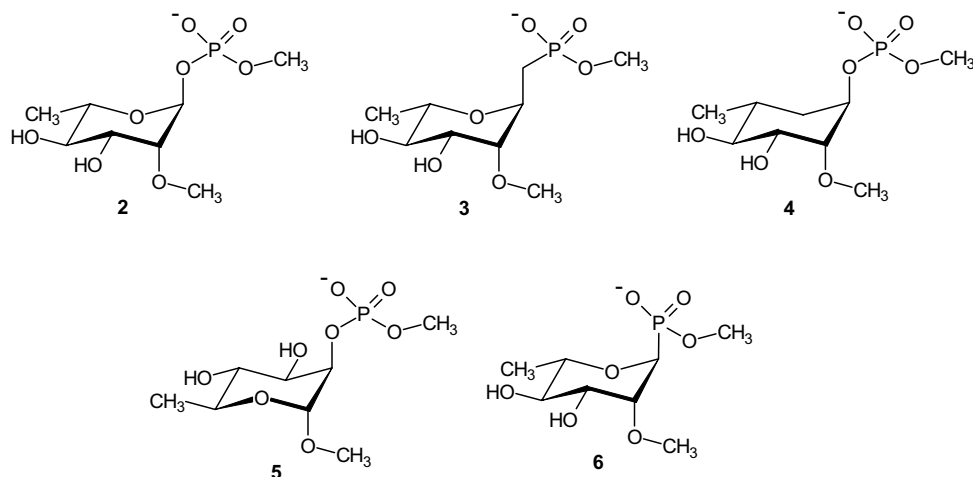
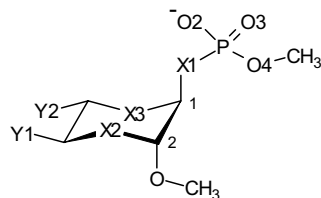


Chart 1.

around the anomeric carbon atom with a methylene group. Looking at the structure of methyl(2-*O*-methyl- α -L-rhamnopyranosyl)-phosphate anion **2** (Chart 1), as the minimum substructure representing the rhamnose moiety in the trisaccharide unit, we devised compounds **3** and **4** as stable mimics. In addition, compound **5** was proposed as a so-called 'inverted' rhamnose unit and compound **6** as a shorter but still comparable compound. In all these cases, the phosphate is no longer an acetalic phosphate but it is either a phosphonate or a phosphate ester. In the attempt to direct the synthetic efforts toward the analogue with the best conformational similarity to the reference compound **2**, we undertook a comparative theoretical study of **2–6** and the results are here reported. In all these structures are present two methyl groups, one mimicking the C-2 substituent of rhamnose and the other the substituent on one oxygen atom of the phosphoryl group.

2. Results

From the formulas in Chart 1 it appears that different numbering schemes should be used for the different compounds. However, in Chart 2 we depicted a single structure representing all compounds **2–6** and refer to it to define the geometrical parameters pertinent to the discussion. For compounds **2–5** the rotation around the 'anomeric' linkage is described by the dihedral angle ϕ ($\phi = \text{X3-C-1-X1-P}$) and the orientation of the phosphate or phosphonate group by the dihedral angles ψ_1 ($\psi_1 = \text{C-1-X1-P-O-4}$) and ψ_2 ($\psi_2 = \text{X1-P-O-4-CH}_3$). The staggered conformations around the bonds just defined are denoted as G, mG, and T. For compound **6** the rotation around the 'anomeric' linkage is described by the dihedral angle ϕ ($\phi = \text{X3-C-1-P-O4}$) and the



	X1	X2	X3	Y1	Y2
2	O	CHOH	O	OH	CH ₃
3	CH ₂	CHOH	O	OH	CH ₃
4	O	CHOH	CH ₂	OH	CH ₃
5	O	O	CHOH	CH ₃	OH
6	-	CHOH	O	OH	CH ₃

Chart 2.

orientation of the phosphonate group by the dihedral angle ψ_1 ($\psi_1 = \text{C-1-P-O-4-CH}_3$). For all the compounds the orientation of the C-2-methoxy group is described by the dihedral angle τ ($\tau = \text{H-C2-O-CH}_3$).

2.1. Compound 2

The literature reports some modeling studies on the conformational properties of dimethyl phosphate and of the sugar-phosphate linkage;^{8–10} ab initio approaches have been used with evaluation of the effect of the solvent. In the present study, the conformational behavior of 1-phosphate **2** was investigated through a DFT approach at the B3LYP/6-31G(d) level.^{11,12} Though a larger basis set could have been chosen, we performed calculations at this level, the smallest acceptable, because of the very large number of starting conformations to optimize (see below).

The linkage of the phosphate group to the anomeric carbon atom of rhamnose results in three internal rotational degrees of freedom associated with the C-1–O-1, O-1–P, and P–O-4 bonds and the corresponding three staggered conformations. Moreover, another degree of freedom is associated with the orientation of the methoxy group at C-2; for it we took into account the two less crowded orientations out of the three possible staggered conformations. A number (54) of starting geometries, all presenting the 1C_4 conformation at the pyranose ring, were prepared and optimized. The balance of steric, stereoelectronic, electrostatic effects is such that not all these structures are minima. Indeed, from the 54 starting conformers a much lower number of minima were obtained. Their energy was recalculated using a continuum solvent model, C-PCM,^{13,14} choosing water as the solvent, to obtain values comparable with water solutions. The results are gathered in Table 1, which reports the energy and some selected geometrical features of the most significant conformers. The procedure was repeated with the same approach starting from geometries with the pyranose ring in the 4C_1 and twisted-boat conformations. Moreover, to get a concise picture of the conformational preferences, the percentage contribution of each conformer of compound **2** to the overall population at 298 K was determined through the Boltzmann equation; then, the percentages relative to a particular arrangement (T, G, mG) at each glycosidic bond and to the orientation of the C-2-methoxy group were added to give the values reported in Table 2.

Thus, calculations on compound **2** predicted that only two of the three staggered orientations about the glycosidic C-1–O-1 bond are populated: the mG and T orientations. The G orientation is not populated as the first conformation presenting this arrangement of the ϕ angle is more than 6 kcal/mol higher in energy than the global minimum. A conformational preference for the mG with respect to the T conformation is observed indicating that compound **2** shows the normal *exo*-anomeric effect.[†] Actually, each of the mG and T orientations consists of a set of conformers that differ in the orientation of the phosphate group, that is in ψ_1 and ψ_2 . A large flexibility appears at these two dihedral angles with all the three orientations populated though the T orientation and the G orientation are preferred at the ψ_1 and the ψ_2 torsion, respectively. The global minimum **2A** (Fig. 1) is representative of all these conformational

preferences as it accounts for a population percentage greater than 50%.

In principle, phosphate **2** has flexibility of the pyranose ring as the other degree of conformational freedom. An inversion of the pyranose ring from the 1C_4 to the 4C_1 chair conformation could allow an equatorial arrangement of the bulky methylphosphate group at C1, though this tendency should be opposed by the anomeric effect. Moreover, twisted-boat conformations might have a stability comparable with the 1C_4 conformation. Actually, conformers of **2** in the 4C_1 or twisted-boat conformations resulted more than 4 kcal/mol higher than the energy of conformer **2A**, indicating that they are energetically disfavored structures.

The above discussion on the stability of the conformers of compound **2** is based on the differences of their energy values. However, when energy differences are small as in this case, entropy effects are important in calculating Boltzmann distributions. We performed frequency calculations on all the minimum energy conformers of compound **2** and obtained, through the thermochemistry analysis, the correction to Gibbs free energy, thus determining their ΔG values. Though some differences in the relative ΔG 's of the various conformers with respect to *E*'s were found, the overall conformational preferences depicted above were substantially unchanged when depicted through ΔG 's. In particular, the preference for 1C_4 versus 4C_1 conformation was maintained; in fact, the most stable 4C_1 conformer showed $\Delta G > 4$ kcal/mol with respect to the most stable 1C_4 conformer. Moreover, the T:G:mG ratios at ϕ (22.6:<0.1:77.4), ψ_1 (52.4:34.9:12.7), ψ_2 (17.5:73.0:9.5) were close to the values in Table 2 as well as the G:mG ratio at τ (72.0:28.0). According to these results and in consequence of the fact that frequency calculations are very demanding in terms of computer resources due to the large number of conformers located for each compound, we did not perform frequency calculations for the analogues **3–6** and thus the following discussion on their conformational preferences is carried out in terms of energy values.

2.2. Compound 3

Calculations on the phosphonate **3** were performed with the same approach reported above for **2** taking primarily into account only the 1C_4 conformation of the pyranose ring. In this case, no conformer accounts for more than 50% of the overall population and a very large number of minima was located (Table 1); however, if we consider only the 1C_4 conformations, the overall behavior of the C1 substituent is not so different to that of **2** (Table 2). The similar percentages of populated intervals at the torsion denoted by ϕ and the fact that all the three orientations at ψ_1 and ψ_2 appear populated though with some differences in the percentages, indicate

[†] It is worth pointing out that the preference for the gauche over the anti conformation was also observed for the model 2-*O*-methylphosphonotetrahydropyran anion when we studied it at the same level of calculations above described: the presence of substituents on the pyranose ring of compound **2** does not appear to exert a significant influence on the behavior of the phosphate group.

Table 1. Torsional angles, relative energies, and equilibrium percentages of the preferred conformations of compounds **2–6**

	Ring conf.	ϕ (°)	ψ_1 (°)	ψ_2 (°)	τ (°)	E_{rel} (kcal/mol)	%
2A	1C_4	−69	−150	72	35	0.00	57.9
2B	1C_4	−154	68	69	27	0.96	11.5
2C	1C_4	−80	−144	72	−52	1.01	10.5
2D	1C_4	−94	89	−155	32	1.52	4.5
2E	1C_4	−148	73	67	−28	1.59	3.9
2F	1C_4	−159	69	−160	30	1.76	2.9
2G	1C_4	−60	−47	−79	35	1.89	2.4
2H	1C_4	−58	−38	110	35	2.02	1.9
2I	1C_4	−105	−93	−170	−48	2.30	1.2
Others							3.3
3A	1C_4	−49	−168	86	−48	0.00	15.7
3B	1C_4	−157	64	84	−39	0.00	15.7
3C	1C_4	−49	−167	−152	−47	0.27	9.9
3D	1C_4	−68	77	90	−48	0.38	8.3
3E	1C_4	−49	−166	−91	−47	0.38	8.2
3F	1C_4	−156	78	−165	−28	0.43	7.7
3G	1C_4	−69	78	−131	−48	0.58	5.9
3H	4C_1	57	−92	122	24	0.62	5.5
3I	1C_4	−72	75	−109	31	0.92	3.3
3J	4C_1	67	103	−177	14	1.00	2.9
3K	1C_4	−178	173	−85	−32	1.05	2.7
3L	4C_1	68	98	86	20	1.22	2.0
3M	1C_4	−47	−170	88	32	1.24	2.0
3N	1C_4	−64	77	91	32	1.34	1.6
3O	1C_4	−48	−168	−152	32	1.60	1.1
3P	1C_4	−45	−47	118	−46	1.62	1.0
Others							6.5
4A	1C_4	−90	−74	−74	34	0.00	25.5
4B	1C_4	−160	68	73	28	0.13	20.3
4C	1C_4	−94	−76	−75	−40	0.60	9.3
4D	1C_4	−94	−72	159	33	0.66	8.4
4E	1C_4	−154	77	73	−24	0.68	8.0
4F	1C_4	−86	−166	73	33	0.72	7.5
4G	1C_4	−157	71	−161	29	0.86	6.0
4H	1C_4	−96	−73	164	−40	1.23	3.2
4I	1C_4	−94	−178	−75	32	1.23	3.2
4J	1C_4	−85	−165	73	−42	1.33	2.7
4K	1C_4	−153	76	−164	−23	1.59	1.7
4L	1C_4	−92	−171	−75	−39	1.78	1.3
Others							2.9
5A	1C_4	−92	−65	−77	53	0.00	26.5
5B	1C_4	−90	−174	73	53	0.01	25.9
5C	1C_4	−77	−47	121	53	0.27	16.9
5D	1C_4	−89	−172	−69	53	0.29	16.3
5E	1C_4	−90	−61	158	53	0.72	7.9
5F	1C_4	−89	−174	168	53	1.06	4.4
Others							1.7
6A	1C_4	−64	86		33	0.00	40.6
6B	1C_4	−64	86		−42	0.39	20.9
6C	1C_4	−56	−79		34	0.74	11.7
6D	$B_{3,0}/{}^4S_0$	−179	82		55	1.22	5.2
6E	1C_4	−57	−80		−41	1.37	4.0
6F	$B_{3,0}/{}^4S_0$	−62	85		54	1.49	3.3
6G	$B_{3,0}/{}^4S_0$	−35	−74		55	1.60	2.7
6H	1C_4	−173	90		35	1.61	2.7
6I	1C_4	−171	−172		32	1.99	1.4
6J	1C_4	−173	93		−41	2.01	1.4
6K	$B_{3,0}/{}^4S_0$	−168	−150		56	2.07	1.2
6L	$B_{3,0}/{}^4S_0$	−59	84		−39	2.15	1.1
Others							3.8

Table 2. Conformational preferences of the glycosidic bonds and of the C2-methoxy group of compounds **2–6** (T: *ap*, antiperiplanar; G: *sc*, synclinal; mG: *–sc*)

	ϕ			ψ_1			ψ_2			τ	
	T (%)	G (%)	mG (%)	T (%)	G (%)	mG (%)	T (%)	G (%)	mG (%)	G (%)	mG (%)
2	20.4	<0.1	79.6	68.8	24.8	6.4	9.5	86.3	4.2	81.6	18.4
3^a	31.9	0.4	67.7	48.3	49.1	2.6	29.0	52.1	18.9	11.0	89.0
4	39.1	<0.1	60.9	16.2	36.1	49.7	19.5	38.8	41.7	72.2	27.8
5	0.2	<0.1	99.8	47.0	1.1	51.9	30.1	26.5	43.4	99.0	1.0
6^a	8.6	0.6	90.8	4.4	77.1	18.5	—	—	—	67.7	32.3

^aPercentages based only on the ¹C₄ conformations.

that the flexibility at the glycosidic torsions found for **3** well reproduces the flexibility of **2**. However, the C-2-methoxy shows a preference for the mG orientation at the τ torsion contrarily to **2** where a preference for the G orientation is observed.

Then, we determined the preferences of **3** in the ⁴C₁ chair conformation of the pyranose ring and found that the chair inversion produces geometries that favorably compare with the ¹C₄ conformations. Though the most populated conformations present the ¹C₄ pyranose ring geometry, the most stable ⁴C₁ conformation **3H** (Fig. 1) is only 0.62 kcal/mol higher in energy than the global minimum **3A**. The percentage contribution of the ⁴C₁ group of conformations to the overall population resulted not negligible (14%) indicating that the replacement of the anomeric oxygen atom with a methylene group determines the loss of the anomeric effect and makes possible an equilibrium between the two chair conformations of the pyranose ring.

2.3. Compound 4

In principle, the carbocyclic compound **4** should present a behavior similar to **3** as, also in this case, the replacement of the pyranose oxygen atom with a methylene group cancels the anomeric effect and could allow the same ring chair equilibration found in **3**. Contrarily to this expectation, every ⁴C₁ conformation results almost 5 kcal/mol higher in energy than the global minimum **4A** making the behavior of the pyranose ring of compound **4** identical to that of compound **2**. As far as the phosphate and methoxy groups are concerned, the usual analysis presented in Table 2 indicates that, in terms of percentages of populated intervals at the torsion denoted by ϕ , the preference for the mG orientation is still maintained. Moreover, also in this case all the three orientations at the ψ_1 and ψ_2 torsions are populated with a more or less uniform distribution of the percentages among the three staggered orientations. The C2-methoxy shows a preference for the G orientation at the τ torsion in agreement with **2** though with a slightly smaller percentage. **4F** is the conformer of compound **4** corresponding to conformer **2A** of the

reference compound. It is the sixth conformer of **4** in order of stability; however, it has an energy only 0.72 kcal/mol higher than the global minimum **4A**.

2.4. Compound 5

The conformational features of compound **5** appear difficult to compare with **2** owing to the structural differences. First of all the ϕ torsion exhibits an almost complete preference for the mG orientation (Tables 1 and 2). An inspection of the geometries (Fig. 1) suggests that this preference could derive from an intramolecular hydrogen bond between the oxygen atoms of the phosphate group and the adjacent hydroxyl group, which ‘freezes’ this degree of conformational freedom. Also the ψ_1 torsion has a freedom limited by the same H bond; only the mG and T orientations are populated while the value of the percentage of the G orientation is close to zero (1%). The ψ_2 torsion presents a high mobility. Finally, the orientation of the C2-methoxy group in **5** should be controlled by the anomeric effect; in fact, the preference for the G orientation is enhanced and becomes almost complete. As in **2**, also in the case of compound **5** the ring chair inversion produces high-energy conformations, more than 5 kcal/mol higher in energy than the global minimum. It is worthy pointing out that conformer **5B**, which is almost isoenergetic with the global minimum **5A**, corresponds to the most populated conformation **2A** of compound **2** if the arrangement of the C-1 and C-2 substituents is considered. However, they differ in the position of the other substituents on the pyranose ring.

2.5. Compound 6

At last, we considered phosphonate **6**. It lacks of an oxygen atom with respect to **2** and consequently it should have a lower flexibility, deriving from one lacking degree of freedom. It is a quite rigid molecule with a prevailing mG orientation at ϕ and a G orientation at ψ_1 . The methoxy group at C-2 has the same preferred orientation presented by compound **2**. The comparison between **2** and **6** should rely, rather than on the torsional angles, on a comparison of the plots of the molecules (Fig. 1) and of the interatomic distances between the

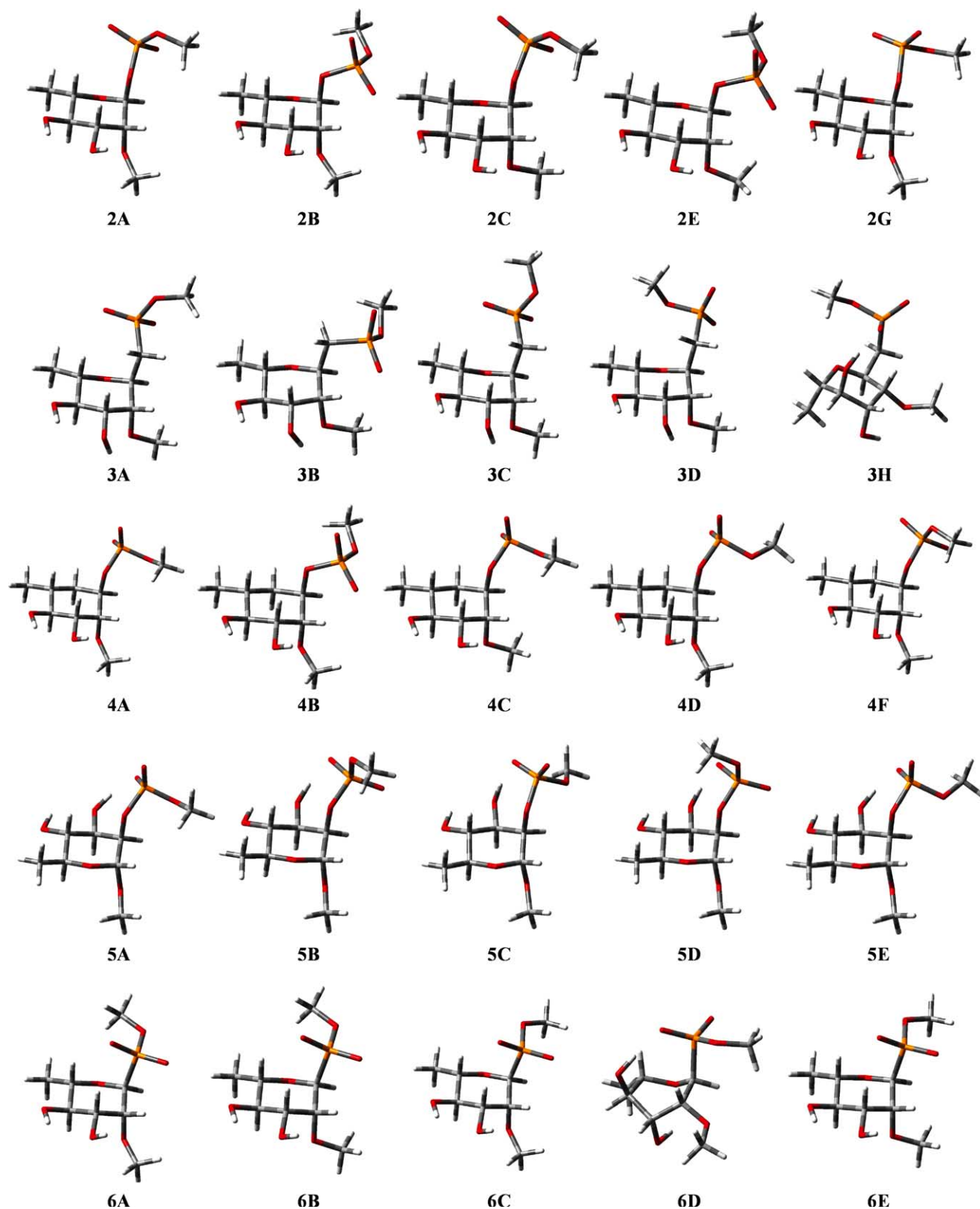


Figure 1. Three-dimensional plot of the most significant conformations of compounds 2–6.

ends of the rhamnose moiety (the methoxy groups) that are relevant for the connection with the other residues of

the trisaccharidic unit (Table 3). Surprisingly, the most populated conformations of **6** are quite similar to some

Table 3. Relevant interatomic distances in the preferred 1C_4 conformations of compound **6** in comparison to compound **2**

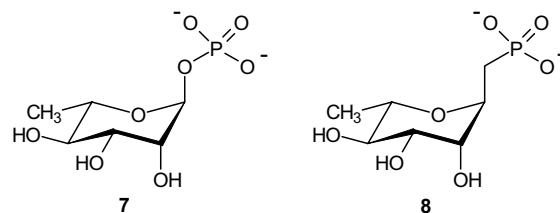
	Me/Me (Å)	O(Me)/O(Me) (Å)
6A	7.09	5.09
6B	6.39	5.16
6C	6.57	5.13
2A	6.62	5.92
2B	6.93	5.05
2E	6.34	5.17
2G	6.38	5.15

of the most populated conformations of **2**; in particular a strict analogy can be observed between **6A** and **2B**, between **6B** and **2E**, between **6C** and **2G** as evidenced by the plots and by the Me/Me and O/O distances. The possibility of ring chair inversion was analyzed also for compound **6** and also in this case the 4C_1 conformation resulted more than 5 kcal/mol higher in energy than the global minimum. Contrarily to all the other cases, twisted-boat conformations (Table 1) resulted populated; they account for about 15% of the overall population.

3. Discussion

The conformational analysis of a set of four mimics of phosphate **2** is here presented in comparison with the reference compound. Compounds **2–6** show several, sometimes tens of populated conformations, so it is more important to evaluate the overall properties of flexibility and mobility than to compare single populated conformations. It is not easy to classify the analogues according to their similarity to **2**; however, some conclusions can be drawn in spite of the fact that it is not known which conformational features should be maintained in order to preserve the activity.

Our calculations predict that the pyranose ring of the substituted rhamnosyl phosphate **2** adopts the 1C_4 conformation. The strong preference for this chair conformation is maintained by the analogues **4** and **5** but not by the phosphonates **3** (1C_4 – 4C_1 equilibrium) and **6** (1C_4 – $B_{3,0}$ equilibrium). This behavior of compound **2** is supported by experimental spectral data of α -L-rhamnopyranosyl phosphate **7** (Chart 3), which shows a value of 1H NMR coupling constant $J_{1,2}$ (1.5 Hz)¹⁵ in agreement with an equatorial–equatorial arrangement of the C-1 and C-2 hydrogen atoms; a contribution of the 4C_1 conformation, in which the same protons are in the axial–axial arrangement, would increase the value of this coupling constant. In the literature is reported the synthesis of the phosphono analogue of **7**, compound **8**;¹⁶ unfortunately, the 1H NMR vicinal coupling constant $J_{1,2}$ of **8** was not measurable. However, the measured values of $J_{3,4}$ and $J_{4,5}$

**Chart 3.**

(both 9.1 Hz) are consistent with a preference for the conformation 1C_4 with a small contribution of the 4C_1 conformation. Moreover, in the synthetic precursor of **8**, which has the hydroxyl and the phosphoryl groups protected, the values of the two same coupling constants are lower (7.5 Hz); this requires a significant contribution of the 4C_1 conformation thus confirming the tendency of this kind of phosphonate to the ring chair equilibration.

The phosphate group of **2** displays a normal *exo*-anomeric effect, that is a preference for the mG over the T orientation at the ϕ angle (mG:T, 80:20). This preference is maintained by **3** (68:32 considering only the conformers in the 1C_4 conformation) and **4** (61:39) and becomes complete in **5** (99.8:0.2). The preference for the G over the mG orientation of the C2-methoxy group in **2** (G:mG, 82:18) appears reverted in **3** (11:89) while it is conserved in **4** (80:20) and **6** (72:28) and enhanced in **5** (99:1).

The present study suggests that the replacement of the anomeric oxygen atom with a methylene group (compound **3**) or its removal (compound **6**) has a heavier influence than the replacement of the pyranose oxygen atom (compound **4**). In fact, compounds **3** and **6** show an equilibrium of the pyranose ring between the two chair conformations or between the 1C_4 and a twisted-boat conformations, respectively, contrarily to the reference compound **2** in which only the 1C_4 conformation is populated. The analogue with the C-1 and C-2 substituents exchanged (compound **5**) is less flexible than the reference compound. It can be concluded that the carbocyclic compound **4** emerges as the best candidate as a mimic of the phosphate **2**, in spite of the fact that it lacks the anomeric and *exo*-anomeric effects. These electronic effects strongly influence the reactivity of carbohydrates as well as the geometry at the anomeric center. In the case of **4** conformational effects probably compensate their absence yielding a compound of geometrical properties close to those of the reference compound. However, it cannot be predicted whether the carbocycle **4**, when inserted into the trisaccharidic unit **1** in the place of a rhamnose residue, will produce oligosaccharide analogues provided of immunogenic activity. In linear polysaccharides the immunological specificity resides primarily in nonreducing terminal

sugar residues;¹⁷ this might suggest a major involvement of N-acetylmannosamine in specific interactions with a minor role of rhamnose. In this respect, modifications in the latter residue have a higher probability to produce analogues with a good profile of activity.

4. Experimental

4.1. Computational methods

An extensive exploration of the conformational space of compounds **2–6** was carried out through DFT calculations using the B3LYP hybrid functional.^{11,12} All the degrees of conformational freedom were considered including rotation around single bonds of the substituents, chair inversion of the pyranose ring, and its conversion to the twisted boat conformation. From the starting geometries, optimized structures were obtained through energy minimizations at the B3LYP/6-31G(d) level performed using the GAUSSIAN98 package.¹⁸ The energies of the conformers so obtained were recalculated with the C-PCM approach^{13,14} as implemented in the GAUSSIAN03 package.¹⁹ Frequency calculations on the minimum energy conformers located for compound **2** were carried out with the same software.

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